EXHIBIT A

Zein-Based Biodegradable Packaging for Frozen Foods.

PADUA, Graciela W., RAKOTONIRAINY, Andrianaivo, and WANG, Qin.

Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 382-D Agricultural Engineering Sciences Building, 1304 W. Pennsylvania Ave., Urbana, IL. Fax: 217 333 9329, e-mail: g-padua@uiuc.edu

Abstract.

This research focuses on the processing of corn zein into flexible, transparent films that may be suitable for packaging of frozen products. Processing involved plasticization of zein with oleic acid. It was added to an ethanolic solution of zein at warm temperatures. The plasticized zein was precipitated out as a soft solid in a stream of cold water. Films were drawn from the soft mass of zein and allowed to dry. Multilayers of dry films were then heated under pressure to produce uniform and transparent films. Films showed relatively high resistance to water, however since they are protein based they absorb water after a period of immersion. Therefore they are not being recommended for long time storage of high moisture products. Instead, they are quite suitable for frozen storage. As such, they would not be expected to come in contact with liquid water for long periods, limiting the opportunity for water uptake. Zein films would be suitable for form-fill-seal processes.

Introduction.

Zein comprises a group of alcohol soluble proteins (prolamines) found in corn endosperm. It accounts for 50% or more of total endosperm protein. Its only known role is the storage of nitrogen for the germinating embryo. Zein can be extracted with aqueous alcohol and dried to a granular powder. Commercial zein is essentially a by-product of the corn wet-milling industry. Centrifugal separation of starch from an endosperm slurry leaves a protein rich mass or corn gluten meal from which zein is extracted. Annual production is over 1 million pounds, mostly used in formulations of specialty food and pharmaceutical coatings. The potential supply of zein, estimated at 750 million pounds per year, calls for expanded markets and drives research and development of novel value-added applications (Shukla, 1992). New methods for extracting zein from dry-milled corn, utilizing in-house produced ethanol and applying membrane technology for solvent recovery, may reduce the cost of zein extraction (Cheryan, 2000).

Film-forming properties of zein have been recognized for decades. They are the basis for its commercial utilization (Winters and Deardorff, 1958; Kanig and Goodman, 1962; Mendoza, 1975; Andres, 1984). Coating films are formed on hard surfaces by covering them with zein solutions and allowing the solvent to evaporate off. The dried zein residue forms hard and glossy protective coatings (Reiners, Wall & Inglett, 1973). Free-standing films or thin sheets, prepared in the laboratory by peeling off dried zein coatings cast on flat surfaces (Aydt, Weller & Testin, 1991), are potentially useful for a range of applications from edible films to biodegradable packaging. Those films are brittle and must be plasticized to make them flexible. Alternatively, films were prepared in the laboratory by drawing them from a moldable, putty-like, resin formed of plasticized zein (Lai and Padua, 1997). Zein resins were also extruded into films (Ha, 1999), seeking to facilitate process scale-up.

The present report will describe the preparation and properties of drawn zein resin films and will present a proposed structural model. It will also discuss the potential application of those films for packaging of frozen foods.

Resin Films.

Zein may be plasticized with long chain fatty acids, while in alcoholic solutions, and later collected as a soft solid mass, after precipitation in cold water. Zein films may be drawn from wet, freshly precipitated resins or formed by extrusion of dry resin pellets. Laboratory preparation of drawn films involves: (1) a plasticization step in which a zein solution in warm aqueous ethanol (70%) is stirred with fatty acids (0.5 - 1 g fatty acid/g zein); (2) a resin formation step, in which cold water is added to the solution to precipitate the plasticized zein; and (3) a kneading step where the precipitate is collected and kneaded into a cohesive and elastic moldable mass. The hydrated resin is very ductile and extensible. Films are drawn from the soft resin, extended over rigid frames, and allowed to dry at room conditions (Lai, Padua & Wei, 1997; Lai and Padua, 1997). Dry films (~0.030 mm thick) are translucent, flexible, ductile, and heat sealable. Under the microscope, they show a fiber network structure with pinholes and structural gaps.

Fusion lamination, performed by heating under pressure (11,000 psi and 100° C) several sheets together, produced transparent sheets that were more ductile and pliable than the original sheets. Rakotonirainy and Padua (1999) suggested that films melted and flowed under the applied pressure filling pinholes and gaps in the structure, thus reducing macro-defects and increasing film uniformity.

Tensile Properties.

Typical TS, E, and Young's modulus values of drawn zein films (1 g oleic acid/g zein) at 25° C and 50% RH are presented in Table 1 (Lai and Padua, 1997). For comparison, TS and E values of collagen films are 3-11 MPa and 25-50%, respectively (Hood, 1987). Typical TS and E values for LDPE films are 9-17 MPa and 500%, respectively (Briston, 1986). Ductility of drawn zein films increased at high RH as expected for protein films. For example, E of zein films (0.5 g oleic acid/g zein) increased from 12 to 30% and Young's modulus decreased from 270 to 150 MPa when RH increased from 50 to 98%, while TS was not significantly ($p>0.05$) affected (Lai and Padua, 1998). This was attributed to zein plasticization by moisture.

Water Absorption.

Sorption isotherms of drawn zein films showed slow water absorption at low values of water activity, a_w. Water was more rapidly absorbed at $a_w>0.85$. Due to their hydrophobic nature, zein sheets resisted wetting. However, prolonged immersion in water resulted in sheet swelling and loss of dimensional stability (Santosa and Padua, 1999). Fatty acid content of sheets affected their water absorption. Sheets containing 0.6 g oleic acid/g zein absorbed 20% water after 24 h of immersion at 25° C while sheets containing 0.8 g oleic acid/g zein absorbed 7% water under the same conditions (Santosa and Padua, 1999). Increased plasticizer levels further decreased water uptake to ~4% at 48 h of immersion.

Property	Value
Tensile Strength	3-5 MPa
Elongation	26 %
Young's Modulus	104 MPa
Water vapor permeability	$0.06\ g \cdot mm/m^2 \cdot h \cdot kPa$

Oxygen permeability	320 x 10^{-14} cm^3 (STP) cm/cm^2.s.Pa
CO_2 permeability	190 x 10^{-14} cm^3 (STP) cm/cm^2.s.Pa
T_g low temperature	- 94 °C
T_α	85 °C

Table 1. Properties of zein resin films.

Gas Permeability.

Water vapor permeability (WVP) of drawn zein films was relatively low (0.06 g·mm/m^2·h·kPa at 25° C and 0/98% RH gradient) (Lai and Padua, 1998) compared to hydrophilic biopolymers such as wheat gluten (2.2 g·mm/m^2·h·kPa at 21° C and 0/85% RH gradient) (Park and Chinnan, 1990) and cellophane (0.3 g·mm/m^2·h·kPa at 38° C and 0/90% RH gradient) (Taylor, 1986). However, WVP of drawn zein films was an order of magnitude higher than that of LDPE films (0.003 g·mm/m^2·h·kPa at 38° C and 0/90% RH gradient) (Smith, 1986). WVP of zein films varied with RH changing from 0.04 to 0.06 g·mm/m^2·h·kPa when RH increased from 50 to 98% (Lai and Padua, 1998). Temperature also affected WVP of drawn zein films with WVP being lower at 25°C than at 5, 15, or 35°C (Lai and Padua, 1998). Polymer films often show an increase in WVP with increased temperature resulting from a higher diffusion coefficient due to increased molecular mobility of plasticizers and polymer chains. However, WVP of zein films also increased at lower temperatures (5 and 15°C). This was possibly due to water condensation on the film surface, which plasticized the protein and increased film WVP (Lai and Padua, 1998).

Drawn films were coated with drying oils (linseed or tung oils) to decrease WVP. Upon exposure to ambient air, drying oils polymerize to form tough, water-repellent coatings. Rakotonirainy and Padua (1999) measured WVP of drawn zein films dipped in laboratory prepared and commercial formulations of tung and linseed oils. Coating films with a commercial formulation of tung oil reduced WVP by ten-fold to 0.005 g·mm/m^2·h·kPa at 25° C and 0/98% RH gradient (Rakotonirainy and Padua, 1999).

Oxygen and CO_2 permeability values of zein films are presented in Table 1 (Rakotonirainy, 2000). For comparison, oxygen and CO_2 permeability values for low-density polyethylene are 22 x 10^{-14} and 94 x 10^{-14} cm^3 (STP) cm/cm^2.s.Pa, respectively. Coating films with tung oil decreases permeability by nearly 25%.

Zein resin films resist freezing temperatures without loosing dimensional integrity. Useo (2000) reported that film samples stored outdoors resisted the freezing-thawing cycles of winter weather. Their tensile properties remained essentially unchanged after frozen storage. A glass transition temperature (Tg) is presented in Table 1 (Lai and Padua, 1997). Also, a high temperature transition point (T_α)is reported.

Structural Model.

Among cereal proteins, those of wheat gluten are the most extensively studied due to their bread-making

properties. Electron micrographs and X-ray measurements on wheat gluten have yielded a structural model (Grosskreutz, 1960 and 1961) that could provide insight on the structure of zein resins. Wheat proteins and phospholipids (7% of gluten) are critical to the structure of wheat gluten. Grosskreutz (1961) proposed that stretched gluten consisted of sheets predominantly parallel to the surface. Each sheet is formed of protein platelets hydrogen-bonded together through an interstitial aqueous phase under the action of hydration and mechanical working. Phospholipids, organized in bilayers, were thought to be located between the platelet sheets acting as slip planes between them.

The structure of zein films was investigated by wide-angle (WAXS) and small-angle (SAXS) X-ray scattering (Lai, Geil & Padua, 1999). WAXS results showed spacings corresponding to the α -helix backbone distance along the chain and the inter-chain spacing between helices. Apparently, film-formation did not change the basic internal structure of the α -helix. SAXS showed a strong periodicity across the film plane for drawn films. Results were interpreted in terms of a platelet structure in drawn films developed during the doughing process and aligned during film formation. Oleic acid seemed to play an important role in the formation of platelet structures. Contrary to phospholipids in wheat gluten films examined by Grosskreutz (1960), oleic acid was present in sufficient concentration (~35%) to develop a periodic protein-fatty acid layered complex throughout the zein film. Granular zein did not show any SAXS periodicity.

Lai, Geil & Padua (1999) proposed a structural model for drawn films based on X-ray measurements. Zein structural prism-like units, consisting of ribbons of folded (antiparallel) α -helical segments, formed protein tetramers. Layers of double-stacked zein units, alternated with bilayers of fatty acid, which conferred flexibility to the films. The structural model suggested that zein plasticization with oleic acid involved dispersion of zein molecules in aqueous alcohol followed by electrostatic adsorption of fatty acids to the charged residues on the zein surface. Addition of cold water during resin precipitation resulted in hydrophobic aggregation of zein-oleic acid units to form sheets. Sheet formation was facilitated by kneading and rolling applied to the resin mass.

Applications.

The potential increase in zein availability has spurred considerable research on its properties and utilization. Intensive research on zein utilization for edible and biodegradable film formulations has resulted in improved performance. Plasticization with fatty acids reduces stiffness and increases flexibility. Heat treatment under pressure improves clarity and texture of sheets. They resist freezing-thawing cycles. Films show relatively high resistance to water. However, since they are protein based they absorb water after a period of immersion. Therefore, they are not being recommended for long time storage in high moisture environments. Instead, they are quite suitable for frozen storage. As such, they would not be expected to come in contact with liquid water for long periods, limiting the opportunity for water uptake. Zein films would be suitable for form-fill-seal processes.

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References.

Andres, C. (1984). Natural Edible Coating has Excellent Moisture and Grease Barrier Properties. Food Process., 45(13), 48-49.

Aydt, T.P., Weller, C.L., & Testin, R.F. (1991). Mechanical and Barrier Properties of Edible Corn and Wheat Protein Films. Trans. ASAE, 34, 207-211.

Briston, J.H. (1986). Films, Plastic. In M. Bakker, The Wiley Encyclopedia of Packaging Technology (pp. 427). New York: Wiley.

Cheryan, M. (2000).

Grosskreutz, J.C. (1960). The Physical Structure of Wheat Protein. Biochim. Biophys. Acta, 38, 400-409.

Grosskreutz, F.C. (1961). A Lipoprotein Model of Wheat Gluten Structure. Cereal Chem., 38, 336-349.

Ha, T.T. (1999). Extrusion Processing of Zein-based Biodegradable Plastics (pp. 166). Ph.D. Thesis, University of Illinois at Urbana-Champaign.

Hood, L.L. (1987). Collagen in Sausage Casings. In A.M. Pearson, T.R. Dutson & A.J. Bailey, Advances in Meat Research (Vol. 4) (pp. 109-129).New York: Van Nostrand.

Kanig, J.L., and Goodman, H. (1962). Evaluative Procedures for Film-forming Materials Used in Pharmaceutical Applications. J. Pharm. Sci., 51, 77-83.

Lai, H-M. and Padua, G.W. (1997). Properties and Microstructure of Plasticized Zein Films. Cereal Chem., 74, 771-775.

Lai, H-M. and Padua, G.W. (1998). Water Vapor Barrier Properties of Zein Films Plasticized with Oleic Acid. Cereal Chem., 75, 194-199.

Lai, H-M., Padua, G.W. & Wei, L.S. (1997). Properties and Microstructure of Zein Sheets Plasticized with Palmitic and Stearic Acids. Cereal Chem., 74, 83-90.

Lai, H-M., Geil, P.H. & Padua, G.W. (1999). X-ray Diffraction Characterization of the Structure of Zein-Oleic Acid Films. J. Appl. Polym. Sci., 71, 1267-1281.

Mendoza, M. (1975). Preparation and Physical Properties of Zein Based Films. M.S. Thesis, University of Massachusetts, Amherst.

Park, H.J. and Chinnan, M.S. (1990). Properties of Edible Coatings for Fruits and Vegetables. ASAE Paper No. 90-6510, St. Joseph, MI: American Society of Agricultural Engineers.

Rakotonirainy, A.M. (2000). Finishing Processes for Zein Biodegradable Films. Ph.D. Thesis. University of Illinois at Urbana-Champaign, Urbana, IL.

Rakotonirainy, A.M. and Padua, G.W. (1999). Effect of Coating and Lamination Processes on Tensile Properties and Water Vapor Permeability of Zein Films. Paper No.11B-25 presented at the Annual Meeting of the Institute of Food Technologists, 24-28 July 1999, Chicago, IL.

Reiners, R.A., Wall, J.S. & Inglett, G.E. (1973). Corn Proteins: Potential for their Industrial Use. In Y. Pomeranz Industrial Uses of Cereals (pp. 285-298). St. Paul, MN: American Association of

Cereal Chemists.

Santosa, F.X.B. and Padua, G.W. (1999). Tensile Properties and Water Absorption of Zein Sheets Plasticized with Oleic and Linoleic Acids. J. Agric. Food Chem., 47, 2070-2074.

Shukla, P.T. (1992). Trends in Zein Research and Utilization. Cereal Foods World, 37, 225.

Smith, S.A. (1986). Polyethylene, Low Density. In M. Bakker The Wiley Encyclopedia of Packaging Technology (pp. 514-523). New York: John Wiley & Sons.

Taylor, C.C. (1986). Cellophane. In M. Bakker The Wiley Encyclopedia of Packaging Technology (pp.159-163). New York: John Wiley & Sons.

Useo, J.-M. (2000). Feasibility of Using Zein-Based Sheets to Protect Hay Bales. M.S. Thesis. University of Illinois at Urbana-Champaign, Urbana, IL.

Winters, E.P. & Deardorff, D.L. (1958). Zein as a Film-Type Coating for Medicinal Tablets. J. Amer. Pharm. Assoc., 47, 608-612.